JOURNAL OF BACTERIOLOGY, June 1982, p. 1314–1321
0021-9193/82/061314-08$02.00/0

Vol. 150, No. 3

Polarity in the *glnA* Operon: Suppression of the Reg$^-$ Phenotype by *rho* Mutations

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Received 7 October 1981/Accepted 21 January 1982

To determine the ability of mutations in *glnA*, the gene for glutamine synthetase (GS), to regulate nitrogen assimilatory enzymes, we assayed histidase and GS in 34 *glnA* (Gln$^-$) strains. Twenty-five *glnA* mutants were RegC, synthesizing high levels of histidase regardless of the availability of nitrogen, and nine were Reg$^-$, synthesizing low levels of histidase in medium containing either limiting or excess ammonia. *rho* mutations were introduced into strains containing *glnA* point mutations or insertions in *glnA*, *glnL*, *glnG*, or *glnF*. The Reg$^-$ phenotype of strains with *glnA* point mutations, but not those with *glnA* or *glnF* insertions, was altered by the presence of *rho*, suggesting that *glnA* (Reg$^-$) mutations are polar and exert their phenotype by decreasing expression of *glnL* and *glnG*. Consistent with this view, no GS protein was detected by two-dimensional gel electrophoresis in *glnA* (Reg$^-$) *rho*$^+$ or *glnA* (Reg$^-$) *rho* double mutants, whereas GS protein was detected in cells of 10 of 11 *glnA* (RegC) strains. Since *glnA* (Reg$^-$) *rho* double mutants synthesize constitutive levels of histidase, GS protein is not necessary for full expression of histidase. Mu d1 insertions in *glnL*, but not those in *glnG*, responded to the presence of a *rho* allele, presumably owing to elevated transcription into *glnG* from the Mu d1 prophage. Our results suggest that *glnA* (Reg$^-$) alleles are polar mutations, and a rho-dependent termination site downstream is postulated as the basis for the polar phenomenon. The data also indicate that, under some circumstances, a significant portion of *glnL* and *glnG* transcription is initiated at the *glnA* promoter.

The field of regulation of bacterial pathways involved in nitrogen metabolism has been dominated for the past decade by a unifying model, that the biosynthetic enzyme glutamine synthetase (GS), the product of the *glnA* gene, serves as a general transcriptional effector. A variety of data lead to this model. Original physiological investigations indicated a correlation between cellular levels of GS and Hut (histidine utilization) enzymes in cells grown with excess and limiting ammonia (3, 22). A causal relationship between levels of GS and synthesis of Hut enzymes was inferred from genetic results: mutants affected in GS levels display concomitant alterations in histidase levels. Mutations that map in *glnA* and result in glutamine auxotrophy (Gln$^-$) alter the regulation of nitrogen assimilatory enzymes in one of two distinct ways. Some Gln$^-$ *glnA* mutants show constitutive production of enzymes normally repressed by ammonia such as the Hut enzymes (RegC). Other Gln$^-$ *glnA* mutants fail to derepress Hut and other regulated enzymes in response to nitrogen limitation (Reg$^-$ [28]). Although the observation of *glnA* (Reg$^-$) mutations is consistent with a regulatory role, it is also consistent with a polar effect exerted by the mutations on the downstream regulatory genes, *glnL* and *glnG* (12, 14, 20). According to this latter view, *glnA* (RegC) mutations result from a high level of transcription, under all conditions of nitrogen availability, from *glnA* into *glnL* and *glnG*.

The observation that merodiploids of *glnA*::Mu *glnG*$^+$ and *glnA*$^+$ *glnG*::Mu were able to induce GS in response to nitrogen limitation, but did not activate histidase expression (20), suggested that transcription from *glnA* into the downstream genes might be necessary for the Reg$^+$ or RegC phenotypes (29). We tested directly the hypothesis of genetic polarity in the *glnA*, *glnL*, and *glnG* regions by examining the effect of mutations in transcription termination factor *rho* on the regulatory phenotype. Mutations in *rho* suppress polarity (for review, see reference 1) and have been isolated by selection for expression of function distal to a polar mutation, for example, in the *lac* (9, 10) and *gal* (5, 6) operons. The effect of *rho* on a number of Mu d1 insertions in *glnA*, *glnL*, and *glnG* were also examined.

Our data indicated that GS is not necessary for maximal expression of the Hut genes; our

results confirmed a prediction of the model of regulation by products of genes downstream from *glnA*. A preliminary report of these findings has been presented (S. K. Guterman, G. Singer, G. Pahel, and B. M. Tyler, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, K153, p. 152).

MATERIALS AND METHODS

Bacterial strains. Bacterial media have been described previously (13). Mutations of *Escherichia coli* K-12 conferring the Gln^- phenotype from the collection of B. Tyler were transferred by cotransduction with *zig-2*::Tn*10* into a genetic background constructed for the purposes of this study. This background, derived from strain DW319 *ilv-340*, *lacZ319*::IS*1* (9, 10), includes the *hutC* genes of *Klebsiella aerogenes* introduced by transduction and selection for use of histidine as the sole nitrogen source. Mutations *rho-15* (5, 6), *rho-115* (9, 11), and *rho-120* (18, 24) were transferred by cotransduction with ilv^+. Additional Gln^- and Reg^- mutations were isolated in strain ET8000 *lacZ319*::IS*1*, $hutC_K$, ilv^+ (14).

Mutations in *rho* were transferred by cotransduction with *ilv-4012*::Tn*10* or *ilv-6960*::Tn*10* (from P. Grisafi), using media containing 15 μg of tetracycline per ml. Generally, the Rho mutant phenotype is scored in *lacZ319* cells as red colony color at 42°C on MacMel agar (MacConkey agar supplemented with melibiose [10]). This phenotype is due to $lacY^+$ expression as a result of transcription past the *rho*-dependent site in IS*1*. Many strains carrying Mu d1 are Lac^+; hence, the Rho phenotype can not be screened directly. *rho* mutations were transferred into Mu d1 strains by cotransduction with *ilv*::Tn*10*, and tetracycline-resistant colonies were scored for rifampin supersensitivity, a characteristic of many *rho* mutant strains (10). Phage P1 was grown on each of several rifampin-supersensitive, putative *rho* isolates and used to backcross *ilv*::Tn*10* into a *lacZ319* strain that does not carry Mud. The *rho* genotype of the donor was scored as a high percentage of red colonies after replica plating of tetracycline-resistant transductants onto MacMel agar containing tetracycline. Two days at 30°C were required for growth of *ilv*::Tn*10* *rho-120* colonies after direct selection for tetracycline resistance.

Enzyme assays. Detergent-treated whole-cell preparations were assayed for GS by the procedure of Pahel et al. (21) and for histidase by the procedure of Prival and Magasanik (23). Glutamate dehydrogenase and glutamate synthase were assayed in sonicated cells by the procedure of Meers et al. (16). β-Galactosidase was assayed by a modification of the procedure of Miller (17); cells were permeabilized by the addition of detergent and $MnCl_2$, as in the histidase and GS assay procedures, rather than by toluene treatment. Enzyme activities were assayed at two or more concentrations of extracts; at least two independent cultures were extracted and assayed for each data point shown.

Radioactive labeling of cells. Cells were grown in 5 ml of minimal medium containing glucose (0.4%), ammonium sulfate (0.2%), and glutamine (0.2%) at 30°C to mid-log phase. The culture was labeled with 20 μCi of [^{35}S]methionine (New England Nuclear Corp.) for 10 min and chased with excess unlabeled methionine for 5 min. Incubation was terminated by chilling on ice, centrifugation, and freezing the cell pellet. The cells were suspended in 0.2 ml of 25 mM Tris-hydrochloride (pH 7.6)–10 mM $MgCl_2$–RNase A (50 μg/ml)–DNase I (300 μg/ml), and extracts were prepared as described by Roberts and Brill (25).

Two-dimensional gel electrophoresis. Reagents (26) and techniques (19) have been described, with the following modifications. The first-dimension tube gels were loaded with 0.1 ml of sample prepared as described above. The slab gels used in the second (sodium dodecyl sulfate) dimension were 1.5 mm thick, and the height of the stacking gel was 1 cm. The gels were run according to the method of Roberts et al. (26). Gels were fixed in 50% trichloroacetic acid for 15 min, rinsed in distilled water, and dried onto Whatman 3MM paper with a Hoefer slab gel dryer. They were placed in direct contact with Kodak X-Omat X-ray film for 3 to 10 days at −70°C.

RESULTS

Determination of regulatory phenotype of *glnA*-linked mutations. A set of 36 spontaneous and mutagen-induced Gln^- strains previously shown to be due to mutations in or linked to *glnA* were assayed for histidase and GS to determine the regulatory phenotype. Enzyme levels for 18 of these mutants are shown in Table 1. Of the 18

TABLE 1. Survey of *glnA*-linked mutations for RegC phenotype

gln allele	Histidase (U/mg)	Reg phenotype
Wild type	0.05[a]	
	0.30[b]	+
glnA2	0.24[c]	C
glnA200	0.05[d]	−
glnA201	0.33	C
glnA202	0.06	−
glnA1854	0.22	C
glnA1855	0.24	C
glnA1856	0.33	C
glnA1857	0.27	C
glnA1858	0.28	C
glnA1859	0.28	C
glnA1860	0.08	−
glnA1862	0.23	C
glnA1863	0.27	C
glnA1864	0.05	−
glnA1865	0.24	C
glnA1867	0.29	C
glnG1866	0.05	−
glnG1870	0.05	−

[a] Growth with excess ammonia; GS activity was 0.15 U/mg.

[b] Growth with limiting ammonia; GS activity was 1.09 U/mg.

[c] All Gln^- strains had 0.01 U of GS activity per mg, except for the *glnA1854* and *glnA1863* mutants, which had about 0.03 U of GS activity per mg.

[d] Mutant cells grown in medium with excess ammonia, glucose, and glutamine.

strains, 12 were found to produce as much histidase when grown with excess nitrogen as did the wild-type strain when grown with limiting nitrogen; i.e., these strains displayed the RegC phenotype. The remaining six strains displayed repressed levels of histidase even under conditions of nitrogen limitation (Reg^- phenotype; data not shown). Table 1 also shows data for two Gln^- strains which were subsequently found to contain *glnG* mutations (14). The regulatory phenotype of 18 additional *glnA* strains was surveyed by assay of histidase. Five strains (carrying mutations *glnA1275*, *glnA1278*, *glnA1286*, *glnA1521*, and *glnA1522*) were found to be Reg^-, and 13 (carrying mutations *glnA1274*, *glnA1276*, *glnA1279*, *glnA1280*, *glnA1281*, *glnA1283*, *glnA1284*, *glnA1285*, *glnA1288*, *glnA1321*, *glnA1322*, *glnA1323*, and *glnA1520*) were of the RegC class. None of the 34 *glnA* (Gln^-) mutants surveyed here displayed normal regulation of histidase (Reg^+); all such mutants were Reg^- or RegC.

Suppression of Reg^- defect by *rho* mutation. *rho* mutations were introduced into *glnA* (Reg^-) strains to determine the effect of polarity suppression on the Reg phenotype. *rho* mutations did alter the Reg^- phenotype in strains carrying alleles *glnA200*, *glnA202*, and *glnA1860* (Table 2); each of the *glnA rho* double mutants now produced derepressed levels of histidase regardless of the availability of nitrogen (RegC phenotype). The allele *rho-120* is most effective in allowing synthesis of histidase; strains carrying this mutation produce four to five times more histidase than do rho^+ isogenic strains. The mutation *rho-15* is least effective in suppressing the polarity of *glnA* Reg^- alleles. The phenotype of the Reg^- mutation *glnA1864* was not altered by the presence of the *rho* mutations studied here. Histidase levels in gln^+ *rho-15*, *-115*, and *-120* were not altered in comparison with rho^+ cells; i.e., repression in the presence of ammonia was not lifted. The RegC phenotype of cells carrying *glnA* alleles *201*, *1862*, *1863*, *1865*, and *1867* was neither restored to a wild-type pattern of repressibility by ammonia, nor was the level of histidase further increased by the presence of a *rho* mutation (data not shown).

TABLE 2. *rho* suppression of the Reg^- phenotype

glnA allele	*rho* mutation	Histidase (U/mg)[a]
Wild type	+	0.05
	15	0.05
	115	0.05
	120	0.04
200	+	0.06
	115	0.12
	120	0.27
202	+	0.05
	15	0.14
	115	0.21
	120	0.28
1860	+	0.07
	115	0.12
	120	0.37
1864	+	0.07
	115	0.06
	120	0.03

[a] Enzyme values are from cells grown with excess ammonia, glutamine, and glucose as the carbon source. Similar enzyme values were obtained with cells grown on limiting ammonia.

***glnA* insertion mutations and *glnF* mutations are not suppressed by *rho* alleles.** Mutations in the general transcriptional release factor *rho* are expected to affect the length of transcripts and efficiency of termination at a large number of sites on the *E. coli* chromosome. Therefore, it is possible that the suppression of the Reg^- phenotype observed here is due to a pleiotropic, nonspecific effect rather than to suppression of rho-dependent termination in the *glnA* region. Several observations argue for the latter interpretation. The Reg^- phenotype of *glnA*::Tn*5* or *glnA*::Mu d1 insertions was not suppressed by *rho* mutations (data not shown). Thus, general physiological perturbation due to *rho* mutation is not sufficient for synthesis of maximum levels of histidase in a *glnA* background. In addition, inability to produce histidase as a result of *glnF208*::Tn*10*, a mutation thought to eliminate a gene product necessary for high-level transcription from the *glnA* promoter (7, 8), was not altered by *rho* mutation. Hence, suppression of the Reg^- phenotype of Gln^- strains by *rho* mutation is limited to a particular class of *glnA* alleles.

Distribution of *glnA* mutations by phenotypic class within the *glnA* gene. The *glnA* mutations surveyed above have been mapped with respect to deletions of the *glnA* gene (14). The distribution of RegC and Reg^- alleles with respect to deletions of the *glnA* gene is shown in Fig. 1. Seven of the eight *rho*-suppressible Reg^- mutations have been mapped to the promoter-proximal portion of the gene, whereas the RegC mutations are randomly distributed. This result is consistent with the interpretation that the *rho*-suppressible *glnA* alleles are polar point mutations, since mutations transcriptionally upstream in a gene tend to exhibit greater polarity than those downstream. The RegC class is presumably composed of nonpolar missense mutations. The Reg^- allele that is not suppressed by any of the *rho* mutations used in this study is located in the promoter-distal deletion interval.

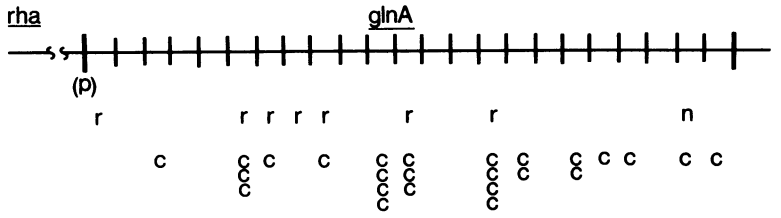

FIG. 1. Distribution of regulatory phenotypes of mutations in the *glnA* gene. Phenotypes were assigned on the basis of histidase production. Each mutation is designated by a letter under the deletion interval (14) in which it was found to map. r, Reg$^-$, *rho* suppressible; n, Reg$^-$, unaffected by *rho*; c, RegC.

Detection of GS protein by two-dimensional gel electrophoresis. The protein spots on two-dimensional gels of crude *E. coli* extracts that comprise adenylylated and nonadenylylated GS have been identified by a number of criteria. Pure reference GS was used to determine the mobility in both dimensions and was mixed with extracts of strains containing *glnA* insertion mutations to demonstrate that there is no alteration as a result of degradation. The GS spots identified in this manner are often altered or missing in *glnA* mutants (see below). The intensity of these spots, observed on gels of extracts of wild-type cells, is severely reduced by growth in the presence of ammonia.

Of 11 *glnA* (RegC) strains analyzed by this technique, 10 produced GS protein. Four of these strains produced GS with wild-type charge, and six produced GS with increased positive or negative charge. The autoradiogram of extracts from cells carrying the *glnA1862* (RegC) mutation is shown in Fig. 2B, and that of wild-type cells is shown in Fig. 2A. The GS spots in *glnA1862* extracts migrated one charge unit to the right in the first dimension, indicating a net increase in negative charge carried by the mutant GS. The GS was substantially overproduced in *glnA1862* cells, as indicated by the difference in exposure of surrounding protein spots for Fig. 2A and B. The presence of the *rho-115* mutation did not further alter the charge of the *glnA1862* mutant GS (Fig. 2C). However, the overproduction of GS observed in *glnA1862 rho*$^+$ cells was not observed in *glnA1812 rho-115* cells. Similar overproduction of altered GS and reduction of overproduction by *rho-115* were observed in other sets of *glnA* (RegC) strains. This phenomenon of remediation of *glnA* (RegC) GS overproduction by the *rho-115* allele has not been further examined.

No GS spots were seen in extracts of four *glnA* (Reg$^-$) mutant strains. Fig. 3A and B illustrate the absence of GS in *glnA202 rho*$^+$ and *glnA202 rho-115* cells, respectively. A similar absence of GS spots was observed with *glnA1860* cells (Fig. 3C) and with *glnA1860 rho-115* cells (Fig. 3D). The presence of GS of wild-type molecular weight in nearly all *glnA* (RegC) strains indicates that the mutations in those strains are nonpolar. Furthermore, the fact that all *glnA* strains that make GS, regardless of the map position or alteration of the protein, are RegC argues that the product of *glnA* is not involved in induction of the other regulated systems. The lack of protein in *glnA* (Reg$^-$) mutants is consistent with the model that these *glnA* mutations are polar.

Regulation of GS, glutamate dehydrogenase, glutamate synthase, and histidase in *rho* mutant cells. The enzymes GS, histidase, glutamate dehydrogenase, and glutamate synthase were assayed in *rho*$^+$, *rho-15*, *rho-115*, and *rho-120* isogenic strains. Full induction of GS was not observed in *rho* mutant cells. For *rho*$^+$, *rho-15*, and *rho-115* cells, GS activity was found to be 1.00, 0.54, and 0.64 U/mg, respectively, in cells grown in medium with limiting nitrogen. In medium with excess nitrogen, all strains produced repressed levels of GS (0.09 to 0.10 U/mg). No differences between *rho* mutant and *rho*$^+$ cells were found for the specific activities of glutamate dehydrogenase, glutamate synthase, and histidase in cells grown in media with excess nitrogen or limiting nitrogen.

***rho* suppression of the Reg phenotype of Mud insertions: arginine as sole nitrogen source.** Five Gln$^+$ strains with Mu d1 insertions in *glnL* or *glnG* were examined for effects of *rho*. Small single colonies were observed on Garg medium in the *rho-115* and *rho-120* mutant derivatives of strains carrying mutations *glnL1101*::Mu d1, *glnL1105*::Mu d1, and *glnL1162*::Mu d1 after incubation for 2 to 3 days at 30°C. The *rho*$^+$ *glnL*::Mud parent strains failed to form colonies even after 7 days on this medium. Strain ET8000 *gln*$^+$ *rho*$^+$ formed large colonies in 1 to 2 days. Neither strains with mutations *glnG1107*::Mu d1, *glnG1150*::Mu d1, nor the *rho* mutant derivatives of these strains grew with arginine as the sole nitrogen source.

These results suggest that the failure of *glnL*::Mud strains to grow on arginine can be overcome to some degree if *glnG* levels are increased. The results of MacNeil et al. (14)

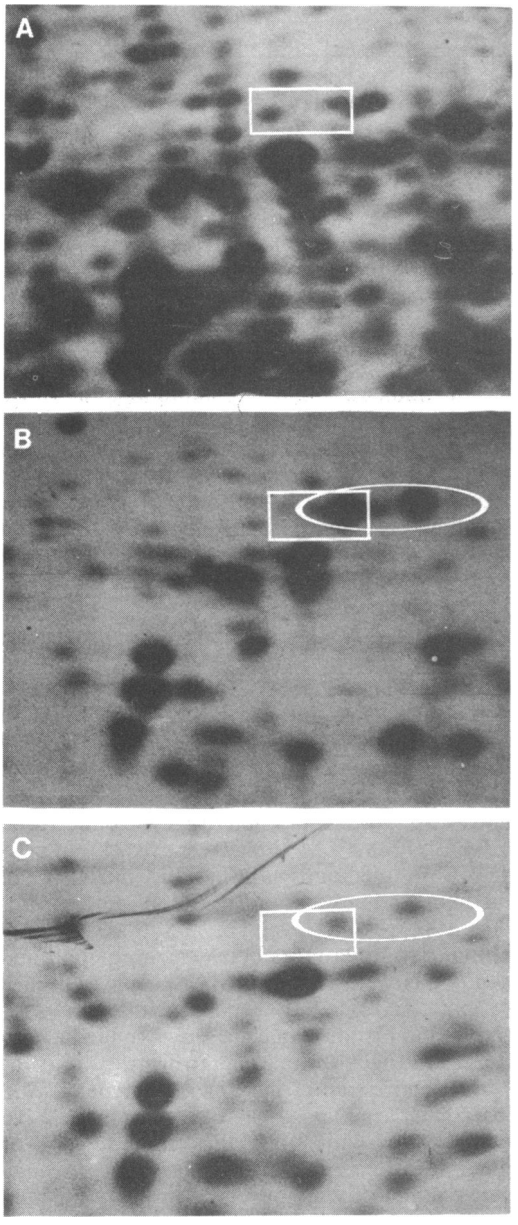

FIG. 2. Two-dimensional gel electrophoresis of wild-type and mutant *glnA1862* (RegC) cell extracts. (A) *gln*$^+$ *rho*$^+$; (B) *glnA1862 rho*$^+$; (C) *glnA1862 rho-115*. The rectangle indicates the location of adenylylated and nonadenylylated GS of wild-type cells; the oval indicates the appearance of mutationally altered GS.

indicate that both polar and nonpolar *glnL* mutants are unable to utilize arginine as the sole nitrogen source. This suggests that, whereas *glnL* product may normally be involved in inducing gene products required for arginine utilization, elevated levels of *glnG* product may partially compensate for absence of *glnL* product in *rho glnL*::Mu d1 strains.

Physiology of Mud fusions in the *glnA* region and the effects of *rho* mutations. The genome of the Mu d1 phage inserts randomly into *E. coli* genes, resulting in inactivation of the function of the affected gene and expression of β-galactosidase at a level that is characteristic of the activity of the promoter that controls the gene into which it has been inserted (4). Mud mutations may perturb regulation of the *glnA* region by several possible mechanisms: direct inactivation of regulatory proteins as a result of insertion into the genes coding for those proteins (4), polarity of the insertion on downstream regulatory genes, and transcriptional restart from a Mud promoter.

In spite of these complexities of interpretation, several observations have accrued from assays of histidase, GS, and β-galactosidase in cells containing Mud fusions in the *glnA* region. The level of β-galactosidase in cells carrying mutation *glnA1049*::Mu d1 was 3,650 U in cells grown with excess nitrogen and 5,700 in cells grown with limiting nitrogen. Similar results were obtained with a *rho-115* derivative. Hence, β-galactosidase is produced in cells devoid of GS (less than 0.01 U) activity, and a small increase in synthesis is induced by starvation for nitrogen. No histidase activation was observed in this strain or in its *rho* mutant derivatives.

Substantial induction of GS was observed in cells carrying the mutation *glnL1162*::Mu d1 in response to nitrogen limitation (Table 3), and this enzyme was induced to an even greater specific activity in the *rho-115* derivative. β-Galactosidase was also induced in these strains in response to nitrogen limitation; however, histidase was not activated in either strain. Cells carrying either of two other Mud fusions in the *glnL* gene, *glnL1101*::Mu d1 and *glnL1105*::Mu d1, displayed induction of GS and β-galactosidase in the *rho* mutant derivatives but induction only of β-galactosidase in the *rho*$^+$ parent. Histidase was activated in none of these strains. In contrast, only basal levels of GS, β-galactosidase, and histidase were observed in cells carrying mutation *glnG1150*::Mu d1 and *rho* mutant derivatives, regardless of availability of nitrogen.

DISCUSSION

We show that the Reg$^-$ phenotype of a class of *glnA* mutations can be altered by the presence of a *rho* mutation. The *glnA* (Reg$^-$) mutations appear to be polar by the following criteria: (i) they fail to complement many mutations in *glnL*, a gene that is transcriptionally downstream (14); (ii) they fail to make detectable GS on gels, a

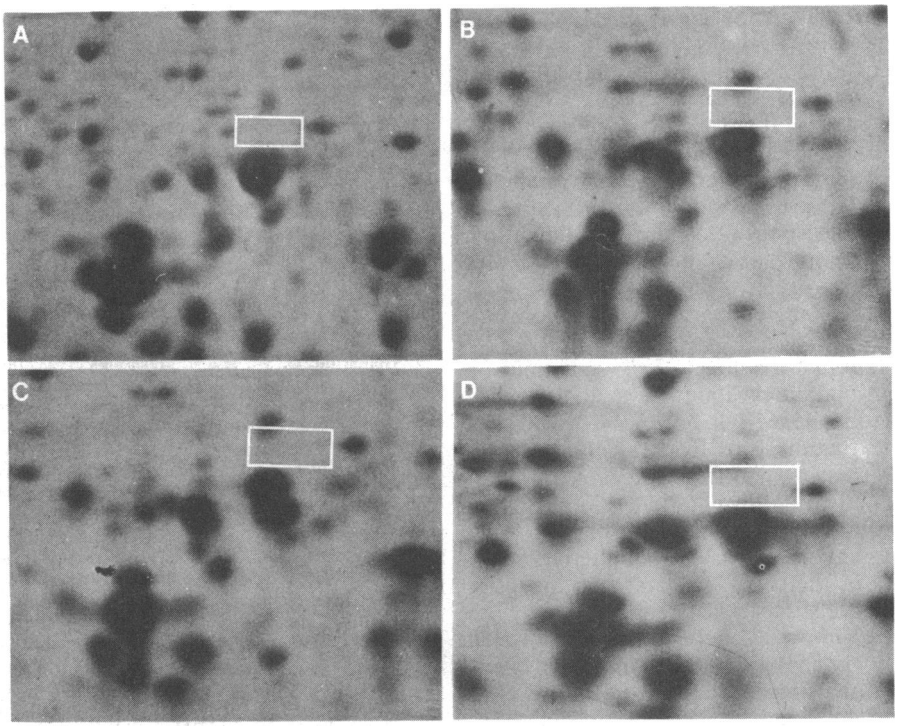

FIG. 3. Two-dimensional gel electrophoresis of *glnA202* (Reg$^-$) and *glnA1860* (Reg$^-$) cell extracts. (A) *glnA202 rho*$^+$; (B) *glnA202 rho-115*; (C) *glnA1860 rho*$^+$; (D) *glnA1860 rho-115*.

result expected of polar mutations but not most nonpolar ones; (iii) they cluster at the promoter-proximal end of *glnA*, where polar effects would be maximized (1); and (iv) the regulatory phenotypes associated with these mutations are suppressed by *rho* mutations. Hence, the *glnA* (Reg$^-$) mutations result in decreasing transcription of genes downstream from the *glnA* promoter.

Other studies have revealed the existence of two genes adjacent to *glnA*. These are identified as *ntrB* and *ntrC* in *E. coli* and *Salmonella typhimurium* (15) and also as *glnL* and *glnG* in *E. coli* (2, 20). Our studies support the model of regulatory functions assigned to products of these genes rather than uniquely to GS protein. The direction of transcription of the *glnA* gene has been determined for *K. aerogenes* (30) and for *E. coli* (2, 13, 27). The *glnA*, *glnL*, and *glnG* genes in *E. coli* are transcribed in the same direction, counter-clockwise on the standard map, consistent with the notion that loss of termination factor rho can affect regulation of these genes.

We show that histidase activation does not require GS, since histidase is synthesized at high levels by cells that carry a *rho* mutation in addition to a Reg$^-$ mutation such as *glnA1860* or *glnA202*. Crude extracts of these strains do not display GS protein by two-dimensional gel electrophoresis. The *glnA* (RegC) mutations that result in constitutive synthesis of histidase are

TABLE 3. Regulation of GS and β-galactosidase by ammonia in *glnL*::Mud and *rho-115* mutant strains

glnL allele	*rho* allele	Enzyme activity					
		GS		β-Galactosidase		Histidase	
		Excess N[a]	Limiting N[b]	Excess N	Limiting N	Excess N	Limiting N
1162	+	0.04	0.79	388	1,216	0.12	0.08
1162	115	0.11	2.09	490	3,040	0.09	0.14
1101	+	0.04	0.04	173	432	0.09	0.06
1101	115	0.06	0.38	79	430	0.12	0.05

[a] Cells grown in medium containing glucose (0.4%), ammonium sulfate (0.2%), and glutamine (0.2%).
[b] Cells grown in medium containing glucose (0.4%) and glutamine (0.2%).

located throughout the *glnA* gene, and strains carrying these mutations display a variety of altered GS proteins. This result is not consistent with the model of histidase regulation exclusively by GS protein. If GS were a positive effector of the histidase gene transcription, one might expect that some, but not all, strains producing an altered GS protein would fail to synthesize histidase in response to nitrogen limitation. However, in spite of dramatic changes in the charge of GS protein, all GS-producing *glnA* mutants surveyed synthesize histidase constitutively.

It is likely that the effect of *rho* mutations on polar *glnA* mutations is not due to an undefined metabolic imbalance but occurs at the level of transcripts that originate from the *glnA* promoter. When transcription from the *glnA* promoter is eliminated by *glnF* mutation, *rho* alleles do not compensate. The polar effects of *glnA*::Mud and *glnA*::Tn*5* insertions are not altered by *rho* mutation. It therefore appears that the *glnA* alleles that are susceptible to suppression by *rho* are polar point mutations, and that suppression of the Reg^- phenotype is due to increased levels of expression of *glnL* and *glnG*, i.e., to relief of polarity.

We propose the following model: any situation that imposes nitrogen limitation will result in elevated transcription from the *glnA* promoter. *glnA* mutants may be limited for nitrogen even though glutamate dehydrogenase is present, if most ammonia is normally assimilated into the cell as a result of GS activity. The phenotype of Gln^- (RegC) mutants results directly from this increased transcription; GS is synthesized, and polarity on *glnL* and *glnG* is not manifested. In the case of Gln^- Reg^- mutants, translation of GS is terminated and polarity occurs. Suppression of polarity by *rho* mutations allows this elevated transcription to extend to the end of *glnA* and into *glnL* and *glnG*. This latter process involves read-through past a rho-dependent termination site.

ACKNOWLEDGMENTS

We thank G. Stave and D. MacNeil for *gln* mutant strains and T. MacNeil for communication of the *gln* fine structure map. G. S. Harrison and S. Uhl provided superb technical assistance. We are grateful to B. Magasanik and the members of his laboratory for the use of their spectrophotometer and for laboratory space.

This study was supported by grants NIH-GM 22527 to B.T., NIH-GM 24461 to S.K.G., and a research award from Merck, Sharp & Dohme to S.K.G.

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